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## Dear Madam or Sir:

Since 1996, we have studied the microbiology of ships' ballast water, ballast residuals, and biofilms within ballast tanks. Our research teams have boarded and sampled more than 150 ships in the Chesapeake Bay and the Great Lakes. We have been funded in these efforts by the Maryland Sea Grant College Program, the National Sea Grant College Program, the US Coast Guard, the US Environmental Protection Agency, the National Oceanographic and Atmospheric Administration, and the Great Lakes Protection Fund. In addition to more than 20 presentations at international scientific meetings, we have published our findings in the peer-reviewed scientific literature and in more popular publications as well.

While there is much to say about microbiology's input in formulating ballast-water discharge standards, we have chosen here only to summarize several issues en route to making recommendations: the presence of fecal-indicator indicator bacteria and human pathogens in ballast tanks, the potential spread of antibiotic resistance via transport of bacteria in tanks, and the size of toxic dinoflagellate cysts.

First, in analyzing samples from ballast tanks, we have detected enteric bacteria *Escherichia coli* and enterococci; the cholera bacterium *Vibrio cholerae*; protozoan parasites *Cryptosporidium parvum, Giardia duodenalis,* and *Encephalitozoon intestinalis*; and the toxic dinoflagellates *Pfiesteria piscicida* and *P. shumwayae*. At present, however, there is no predicting the presence of these pathogens with respect to ballast-exchange practices, time of year the samples were collected, or previous ports of call. In formulating ballast-water discharge standards, therefore, it would be prudent to regard all ships as potential carriers of pathogens.

Second, our analyses of *Vibrio cholerae* have revealed some degree of antibiotic resistance in 76% of isolates from ballast tanks sampled in the Great Lakes and Chesapeake Bay. Antibiotic resistance in bacteria is, of course, an undesirable trait having significant publichealth ramifications. We are testing the hypothesis that antibiotic resistance is imported into Chesapeake Bay via ballast-water operations. Potentially exacerbating the situation is a well-known process whereby bacteria transfer genes to one another. If genes encoding for antibiotic resistance were transferred in ballast tanks, then it would amplify risks associated with ship-mediated transport of bacteria and their subsequent release into receiving ports.

Third, toxic dinoflagellates (and other harmful algae) affect human health and fisheries resources on a global scale. Resting cysts of dinoflagellates readily accumulate in ballast tanks, where they may remain viable for months or years. Indeed, diverse and abundant populations of cysts have been found in tank sediments worldwide. These resting cysts are highly resistant to numerous chemical and other treatments, represent a significant risk of introduction, and clearly should be considered in discussion of ballast-water discharge standards. In particular, appreciating the size of resting cysts (harmful forms range from 10 to  $87 \mu m$ ) is fundamental to setting a scientifically defensible size-exclusion standard.

We recommend the development of discharge standards that exclude, kill, or inactivate potentially harmful microorganisms. Sterilization of ballast water, however, is an unrealistic goal. Instead, we support an approach in which interim and final size-exclusion standards are set in a manner to coordinate with--indeed stimulate--available and emerging treatment technologies. Finally, we concur with the precept that discharge standards should be predicated on the concentration of organisms released into receiving waters and not on their percentage decreases following treatment.

Sincerely,

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